GENETIC STUDY OF *MGMT* GENE PROMOTER METHYLATION

Glioblastoma Multiforme (GBM) is the most malignant subtype of glioma, and the most common primary brain tumor in adults. It is a tumor with a poor prognosis, with an average survival of 15 months, and 24 months in 26-33% of patients (1). The incidence of GBM peaks at around 50 years of age, and is higher in men. The tumor is located in the subcortical white zone , more frequently in the temporal and frontal lobe. It is usually intraparenchymal with an epicentre in the gray matter.

The treatment of choice is maximum surgical resection, followed by radiotherapy plus concomitant and maintenance temozolomide (TMZ) (2). TMZ is a chemotherapy drug that is given for a period of time ranging from 6 weeks to 14 months (treatment plus maintenance). Approximately 35% of the drug crosses the blood-brain barrier . It has been described that the response associated with TMZ is higher in patients who have the *MGMT* gene promoter methylated.

The *MGMT* gene is located on chromosome 10 (10q26). It encodes the enzyme O^6-methylguanidine-DNA methyltransferase, which has a DNA repair function, transferring alkyl groups from the O^6 position of guanine and preventing the formation of bonds in the genetic material. This enzyme inhibits the apoptosis induced by therapeutic alkylating agents in front of DNA tumor.

The methylation of some genes involves the silencing or non-expression of their information. In this sense, the silencing of the *MGMT* gene through the methylation of its promoter is associated with the loss of gene expression, and therefore with a decrease in the DNA repair activity of the enzyme it encodes. We know that the linkage between cytosine and the methyl group (CH3-) (Fig 1) prevents the expression of the associated gene.

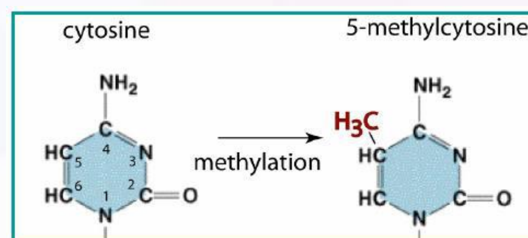


Fig 1. Methylation of cytosine to 5-methylcytosine

The expression of the *MGMT* gene is linked to a greater resistance to therapy with alkylating agents, extremely reactive molecules that cause cell death by binding to DNA. In contrast, their methylation, or lack of function, allows these therapeutic agents to act more effectively (Fig 2).

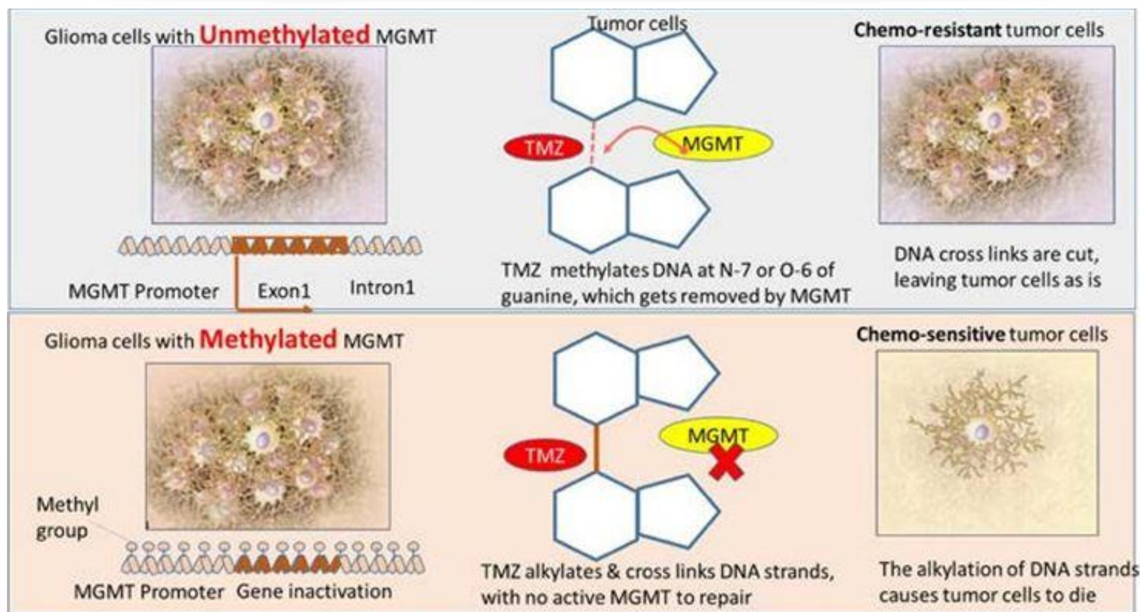


Fig 2. Chemotherapy sensitivity mechanism (TMZ) resulting from the epigenetic inactivation of the *MGMT* tumor repair gene .

Of all the known biomarkers for the diagnosis of GBM, there are 3 that are used in the genetics laboratory routine (3).

Up to now, in Catlab we have been studying two of these molecular biomarkers, related to GBM, in two different tests:

- Genetic study of IDH1 and IDH2 gene variants.
- Study of the 1p/19q codeletion .

Recently, we have incorporated the study of *MGMT* gene promoter methylation. This study is performed with the Easy PGX ready MGMT kit (Diatech). It is a test that allows a qualitative detection by real-time PCR and melting curves of the methylation status of 12 CpG islands located in the promoter of the *MGMT* gene. CpG islands are regions of DNA rich in Citosine and Guanine, normally located in the promoter regions of the gene, where methylation usually takes place.

This test is performed on paraffin-embedded tissue samples.

First, DNA extraction is performed using an automated extractor (MagCore nucleic acid extractor-RBC Bioscience), with the Magcore Genomic DNA FEPE kit, specifically designed to work with paraffin-embedded samples.

Magcore Nucleic Acid Extractor .

Once the DNA is extracted, it is quantified using the molecular spectrophotometer DS-11 (Denovix), which allows us to determine the concentration and quality of the sample obtained.

DS-11 Spectrophotometer Denovix

This DNA is then processed with sodium bisulphite, which transforms unmethylated Cytosines into Uracils. During amplification, Uracil is replaced by Thymine. Methylated cytosines remain unchanged (4) (Fig. 3)

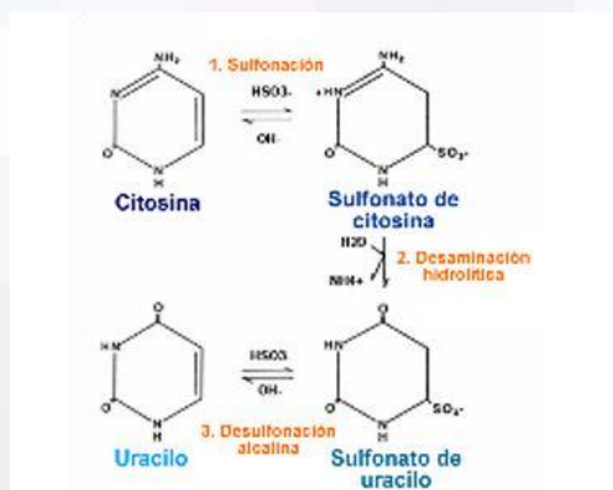


Fig. 3. Cytosine processing with bisulfite.

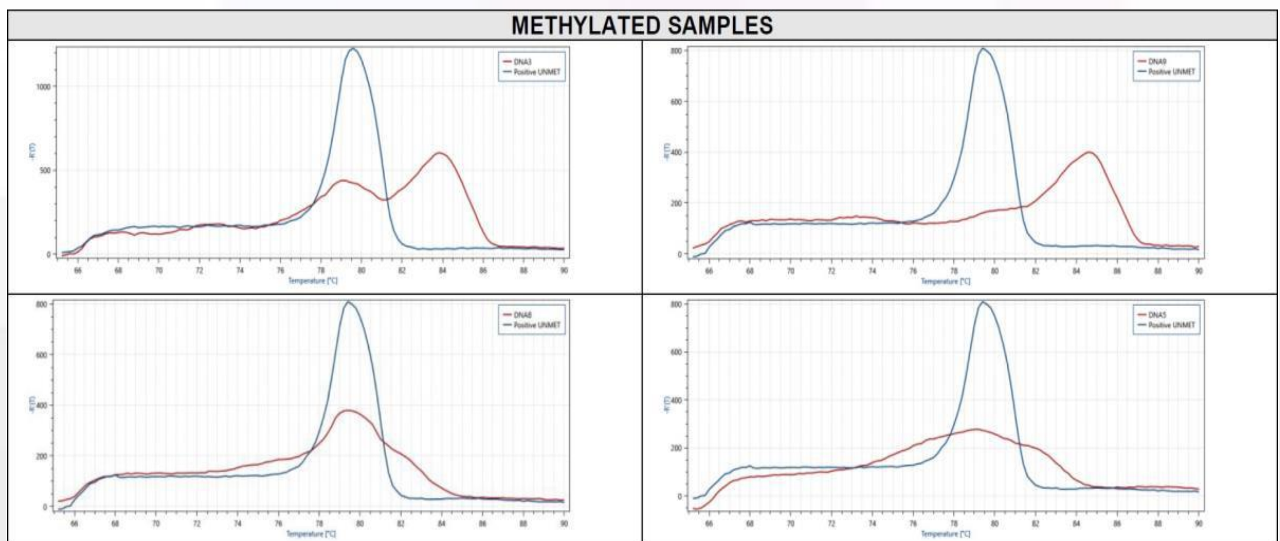
Bisulfite-treated DNA is amplified by methylation-sensitive real-time PCR (MSP) using the Easy PGX kit (Diatech)

Easy PGX (Diatech)

This PCR uses specific primers to amplify methylated and unmethylated alleles. After the melt curve analysis, the methylated and unmethylated samples produce distinct melting peaks, due to their different thymine and cytosine composition..

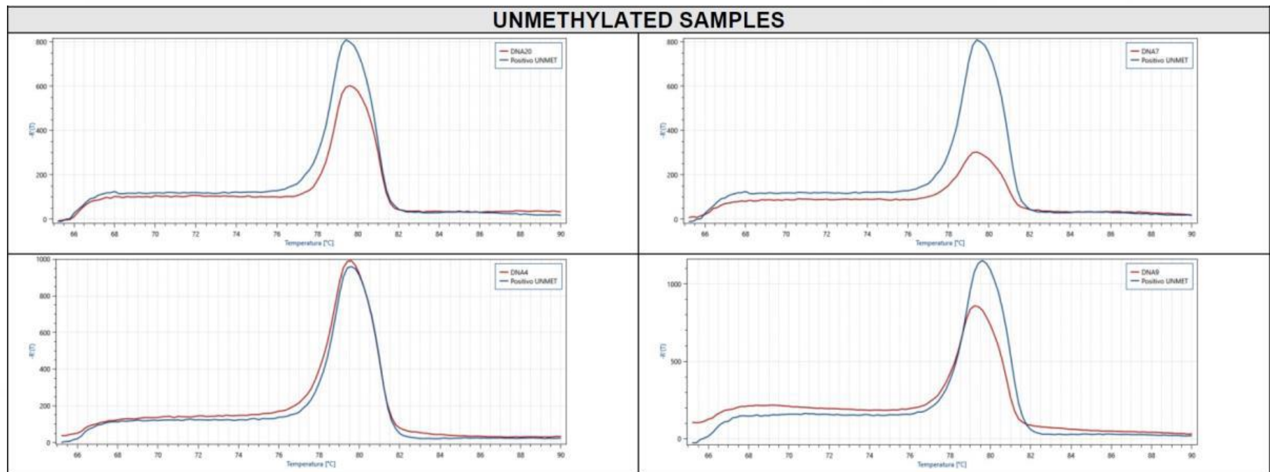
Example of results:

Methylated samples:



The blue curve represents the unmethylated control with a melting temperature of approx . 80ºC. The red coloured methylated samples have a melting temperature of approx. 84ºC.

Non-Methylated Samples:



The melting temperature of the unmethylated samples matches with the unmethylated control (−).

The determination of the *MGMT* gene promoter methylation status enables the selection of those patients with GBM, who are more likely to benefit from treatment with alkylating agents such as Temozolamide and therefore have a more favorable prognosis. Those patients who do not have a methylated *MGMT* gene promoter correspond to a therapy-resistant phenotype, which is a negative prognostic predictor.

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